

Research Article

CHITOSAN-SODIUM ALGINATE BIODEGRADABLE INTERPENETRATING POLYMER NETWORK (IPN) BEADS FOR DELIVERY OF OFLOXACIN HYDROCHLORIDE

PREETI V KULKARNI^{1*}, JATHI KESHAVAYYA²¹SET's College of Pharmacy, Dharwad, Karnataka, India, ²Department of Studies in Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga, Karnataka, India, Email:pritikulkarni1@rediffmail.com

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ABSTRACT

An inexpensive and simple method was adopted for the preparation of chitosan sodium alginate IPN (Interpenetrating Polymer Network) beads for the release of an antibiotic, Ofloxacin Hydrochloride. The polymers were combined at different ratios and the exposure time to the crosslinking agent, glutaraldehyde (GA) was varied to get the beads with different processing parameters. They were evaluated for the size, degree of swelling, drying rate, drug entrapment efficiency and release rate. The absence of any chemical interaction between drug, polymer, and the crosslinking agent was confirmed by Fourier transform infrared (FTIR) spectroscopy. The beads characterized by optical microscopy indicated that they were in the size range of 30 - 250 μ and scanning electron microscopy (SEM) studies were carried out to reveal their surface characteristics. The degree of swelling as well as rate of drying of beads were influenced by the incorporation of alginate into chitosan and the time of exposure to the crosslinking agent. The encapsulation efficiency varied between 76-86 % for different formulations. According to the *in vitro* dissolution studies, the IPN beads showed sustained effect up to 24 hrs; increase in the alginate content and exposure time to the crosslinking agent resulted in reduction of extent of drug release.

Keywords: Chitosan, Sodium alginate, Crosslinking, IPN ((Interpenetrating Polymer Network)

INTRODUCTION

Controlled drug delivery systems offer some advantages compared to the conventional dosage forms, which include reduced adverse reaction, toxicity and frequency of dosing with improved efficacy, patient compliance and convenience¹⁻². Using the novel microencapsulation techniques and varying the polymer ratio and molecular weight, microspheres can be developed as an optimal drug delivery system which provides the desired release profile. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents³.

Microencapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature capsules that can release their contents at controlled rates under specific conditions⁴. The choice of materials and the methodology used for encapsulation are dependent on the active agent in question and the target application⁵. The importance of polymeric blends has increased in recent years because of the preparation of the polymeric materials with desired properties, low basic cost, and improved processability⁶. The Interpenetrating polymer networks (IPNs) have found astonishing applications in the CR of bioactive molecules⁷. IPN is a polymer comprising two or more networks, which are at least partially interlaced on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken⁸.

The use of natural polymers as drug carriers has received much attention in the pharmaceutical field due to their safety. In particular, the polysaccharides such as sodium alginate and chitosan have been studied for application in the design of dosage forms for controlled release⁹. The use of natural polymers is valuable based on proven biocompatibility. Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin¹⁰⁻¹¹. Properties of chitosan make the polymer suitable for use in biomedical and pharmaceutical formulations¹²⁻¹³. It has also been used for the encapsulation of drugs¹⁴⁻¹⁹. Sodium alginate is a natural and hydrophilic polymer suitable for the entrapment of water soluble drugs²⁰⁻²².

Ofloxacin hydrochloride was chosen as a model drug for encapsulation in the polymer matrix and to study the *in vitro* release. Although many drugs have been extensively investigated using natural polymeric carriers, the studies on the release of antibiotic drugs are limited. Ofloxacin HCl is a synthetic

antimicrobial having a quinolone structure which is active primarily against gram-negative bacteria, but is comparable to Ciprofloxacin for gram-positive organisms and certain anaerobes²³. Formation of interpolymer complexes of chitosan with sodium alginate and development of IPN beads by crosslinking using glutaraldehyde (GA) has been discussed in this study.

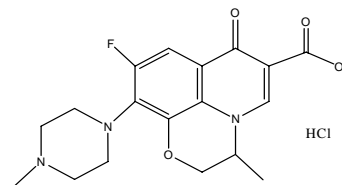


Fig. 1: Chemical structure of Ofloxacin hydrochloride

MATERIALS AND METHODS

Materials

The gift sample of Ofloxacin HCl was supplied by Sun Pharmaceuticals Pvt. Ltd., Mumbai, India. The 75-85 % deacetylated chitosan with a viscosity of 200-800 cP measured by Brookfield viscometer in 1% w/v of chitosan solution, in 1 % v/v acetic acid solution was purchased from Aldrich Chemical Company, USA. Sodium alginate, glutaraldehyde solution (25 % v/v) and all chemicals were purchased from S.D. Fine-Chemicals Ltd. Mumbai, India and used as received. Distilled water was used throughout the study.

Method of preparation of IPN beads²⁴

The IPN beads of chitosan and sodium alginate crosslinked with GA were prepared by using the documented procedure with some modification. Solutions of chitosan and sodium alginate were prepared separately in 1 % v/v acetic acid solution and distilled water respectively, by stirring on a magnetic stirrer, at 40 °C. Varying proportions of polymers were mixed, as shown in Table 1. The final concentration of mixture of polymers in each solution was 3 % w/v. Weighed quantity of Ofloxacin. HCl (40 % w/w of dry weight of polymer) was added and stirred further for 30 min. A

number of formulations were prepared by extruding a solution of chitosan alone, and in combination with sodium alginate, using a syringe into a solution of methanol containing 1% GA and 0.1 ml conc. HCl. The temperature (40 °C) and speed (2000- rpm) were maintained constant. The contact time with crosslinking agent was varied such as 15 min and 30 min for different formulations. The beads were recovered by filtration, washed with methanol, followed with distilled water and dried in an oven at 40 °C. The yield of beads thus formed was calculated and the formulations are coded as shown in Table 1. Experimental conditions such as distance between container and syringe, and number of drops per minute were maintained.

Evaluation

Drug polymer interaction study by FTIR spectroscopy

FTIR spectral data were used to confirm the intactness of drug molecule in the formulation and to know any chemical interaction between polymer, any other additive, crosslinking agent, drug or the chemical. The IR spectral data were taken on a Nicolet, Model Impact 410, USA. About 2 mg of the samples were ground with spectroscopic grade KBr and the pellets were prepared under a hydraulic pressure of 600 kg/cm². The spectra were obtained by eliminating the background noise and scanning was done in the range of 4000-500 cm⁻¹. The instrument uses a He-Ne laser (632.8 nm) as an equipment carrier with deuterated triglycine sulfate (DTGS) detector. The laser is used as an internal calibrator because it emits light at the precise calibrated points that generate wavenumbers with an accuracy of ± 0.01 cm⁻¹ and controls the moving mirror's position and triggers the capture of data.

Measurement of bead size²⁵

Particle size of the beads was determined by optical microscopy. Average of 100 beads were used for the study and the mean particle size (arithmetic mean diameter) was considered to be the deciding factor in selecting optimum formulation conditions for each variable parameter studied.

Scanning Electron Microscopy (SEM)

SEM was used to obtain the information on topography and surface characteristics of beads. The sample was deposited on a brass hold and sputtered with gold. The micrographs were obtained with JSM 6400 scanning microscope (Japan) at the required magnification. The working distance of 39 mm was maintained and 15 KV of voltage was used, with the secondary electron image (SEI) as a detector.

Swelling study²⁶

Randomly selected samples of beads were soaked in water and at a fixed time interval (an hourly interval), they were taken out and blotted carefully (without pressing hard) to remove the surface adhered water. These swollen beads were weighed using an electronic balance until a constant weight was achieved and the rate of water uptake was calculated with respect to the initial dry weight of the samples. In order to maintain the accuracy, the experiments

were carried out in triplicate (n=3). The percentage swelling of microspheres was calculated using formula.

Drying study²⁷

The beads with nearly equal initial mass were used for the test. The beads formed were dried at 40 °C and weighed at an hourly interval of time, until a constant weight was achieved. Mass measurements were done on a single pan analytical balance. In order to maintain the accuracy, experiments were carried out in triplicate.

Encapsulation efficiency²⁸

The encapsulation efficiency of drug was determined by incubating the known mass of beads with 5 ml of water, till the beads were swollen completely. The swollen beads were crushed using a mortar and pestle and the solution thus formed was sonicated for 2 min using 60 MHz frequencies. The above solution was concentrated using a rotary flash evaporator to form a thick paste, to which about 10 ml of methanol was added to extract the entire drug. The precipitated polymer was separated by centrifugation. After appropriate dilution, the absorbance of the solution was measured at 280 nm and the drug content was determined.

In vitro dissolution study²⁸⁻³⁰

Dissolution studies were carried out in 900 ml simulated gastric fluid (SGF, 0.1 M HCl, pH 1.2) and simulated intestinal fluid (SIF, phosphate buffer, pH 7.5), both without enzymes, prepared according to the US Pharmacopeia using the dissolution tester equipped with eight pedals. The dissolution rates were measured at 37 °C and 100-rpm speed. A 10 ml of aliquot was withdrawn from the vessel at predetermined time intervals (every 30 min) and replaced with an equal volume of corresponding dissolution medium. The samples were diluted appropriately before the assay. The amount of drug released was monitored by measuring the UV absorbance at 280 nm and concentration of drug was determined using the calibration curves constructed from reference standards.

RESULTS AND DISCUSSION

Polymer, drug as well as the formulations were characterized by FTIR spectroscopy to know any possible interaction between drug, polymer and the crosslinking agent (Fig 2). The FTIR spectrum of chitosan showed peaks corresponding to O-H stretching at 3431 cm⁻¹ and amine group (NH₂) stretching at 2925 cm⁻¹ respectively. Sodium alginate showed peaks at 3427 cm⁻¹ due to O-H stretching and at 1742 cm⁻¹ corresponding to C=O stretching of carboxylic group respectively. The drug ofloxacin showed a broad band at 1633 cm⁻¹ due to C=O stretching of carboxylic group. The aromatic C-H stretching band was observed at 2844 cm⁻¹. The spectra of drug loaded IPN displayed peaks due to imine (C=N) formation (crosslinking of chitosan with GA) at 1579 cm⁻¹ and acetal formation (crosslinking of sodium alginate with GA) at 1096 cm⁻¹, along with peaks due to the presence of drug at 1628 cm⁻¹ (aromatic keto group) and 1728 cm⁻¹ (C=O stretching of carboxylic group) respectively. This denotes the drug was intact in the formulation and did not react either with the polymer or the crosslinking agent.

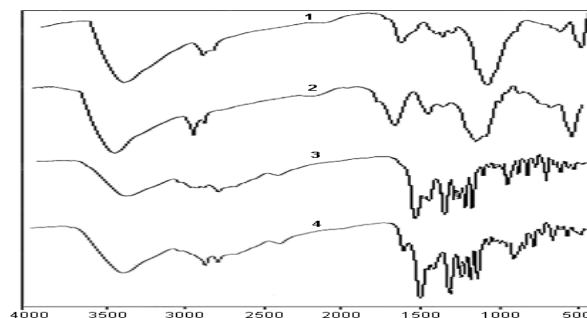


Fig. 2: FTIR spectra of (1) chitosan, (2) sodium alginate, (3) Ofloxacin HCl, and (4) Ofloxacin HCl loaded chitosan - sodium alginate IPN crosslinked by GA

Table 1: Evaluation of chitosan - sodium alginate IPN beads

Formulation code	Polymer	Time of exposure to GA (min)	Yield %	Mean particle size (μ)	Encapsulation Efficiency %
F1	Ch	15	92.15 \pm 1.72	212 \pm 0.19	79.40 \pm 1.7
F2	Ch	30	93.15 \pm 1.66	216 \pm 0.6	76.94 \pm 1.3
F3	Ch + (10%) SA	15	92.14 \pm 2.14	222 \pm 0.12	84.38 \pm 1.73
F4	Ch + (10%) SA	30	94.0 \pm 1.6	196 \pm 2.1	82.14 \pm 0.9
F5	Ch + (30%) SA	15	91.65 \pm 2.19	156 \pm 0.8	85.50 \pm 0.96
F6	Ch + (30%) SA	30	93.66 \pm 2.3	222 \pm 0.4	84.81 \pm 2.06
F7	Ch + (50%) SA	15	92.90 \pm 1.7	219 \pm 1.0	80.85 \pm 0.6
F8	Ch + (50%) SA	30	92.4 \pm 2.8	236 \pm 0.43	79.90 \pm 0.8

Where Ch – Chitosan SA – Sodium alginate. The values are represented as mean \pm standard deviation

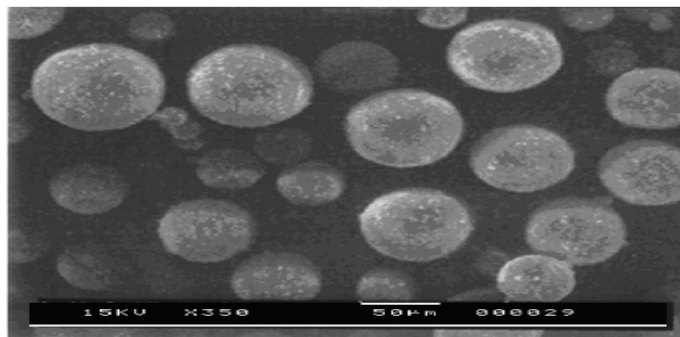


Fig. 3: SEM view of chitosan-alginate IPN beads

The method of preparation allowed the beads smaller than 250 μ m (Table 1). It was observed that the particle size did not vary significantly either by altering the polymer ratio or variation in the exposure time to GA. The beads were further evaluated by SEM (Fig 3), to know their morphology. The beads were found to be spherical in shape and ofloxacin was successfully encapsulated into them.

The transport of water through the polymer depends upon its rigidity and extent of crosslinking ability. The results of water uptake by the beads are displayed in Fig 4 and 5, which indicate that

all the beads absorb maximum amount of water during the first hour. The beads prepared by 15 min exposure to GA, absorbed more amount of water than those prepared by extended exposure time to GA (30 min). Plain chitosan beads, both crosslinked for 15 min and 30 min, showed lesser extent of swelling compared to those of alginate incorporated beads; sodium alginate being a hydrophilic polymer. The increased porosity and decreased crystallinity of the polymer enhance the water uptake and swelling ability. Crosslinking occurs fast at higher temperature and highly crosslinked beads absorb very small amount of water.

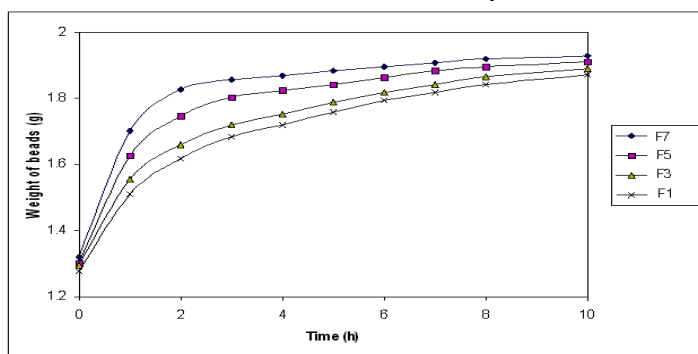


Fig. 4: Rate of water uptake by beads (exposed to GA for 15 min)

In order to optimize the drying conditions, some of the beads with different process variables were selected with approximately equal initial mass. The results of drying (Fig 6 and Fig 7) indicated that the time of exposure to GA influenced the drying rate of the beads. The beads crosslinked for 15 min were dried faster (20 hrs) as compared to the beads crosslinked for 30 min (24 hrs), which is due to an increased rigidity of the polymer by increased crosslinking. Incorporation of sodium alginate led to slower drying which is due to hydrophilic nature of the polymer.

The production yield was relatively high for all the formulations and processing parameters did not affect it much. The encapsulation

efficiency of beads prepared in our study varied between 76 % - 86 % as shown in Table 1. Incorporation of sodium alginate in the matrix and reduced contact time with the crosslinking agent has produced beads with higher entrapment efficiency. The chitosan-alginate crosslinking leads to a three dimensional lattice structure entrapping the drug. Chitosan beads entrapped 79 % of drug, and as the IPN was formed the entrapment efficiency increased up to 85 %, which reflects the composition and space available within the matrix. The porosity of alginate beads is responsible for release of small amount of drug such as during washes and explains the low encapsulation efficiency as further increase in alginate content in IPN beads.

Concentration of alginate was an influential factor: addition of alginate (10 %) and exposure to GA for 15 min and 30 min showed 84 % and 82 % entrapment respectively. On increasing the alginate concentration to 50 % the efficiency was reduced to 80 % and 79 % (exposed to GA for 15 min and 30 min) respectively. This might be due to loss of drug during washing from a hydrophilic polymer matrix.

In order for the microencapsulated drug to elicit a response, it must be released from the beads. Therefore the release profile of Ofloxacin from chitosan-sodium alginate IPN beads was evaluated. The release profile was characterized by an important initial burst effect followed by a continuous and fast release of drug from beads containing more amount of alginate. The highly porous structure of alginate beads explains this fast release pattern³¹.

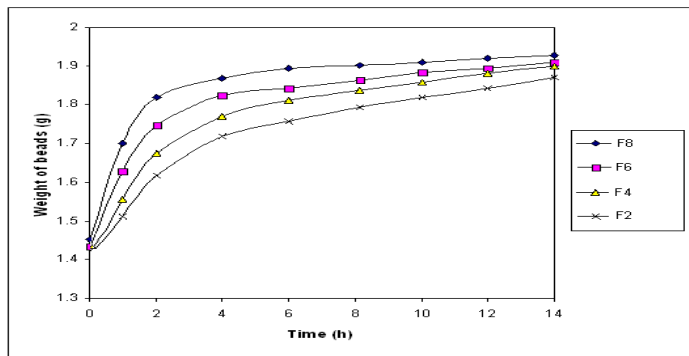


Fig. 5: Rate of water uptake by beads (exposed to GA for 30 min)

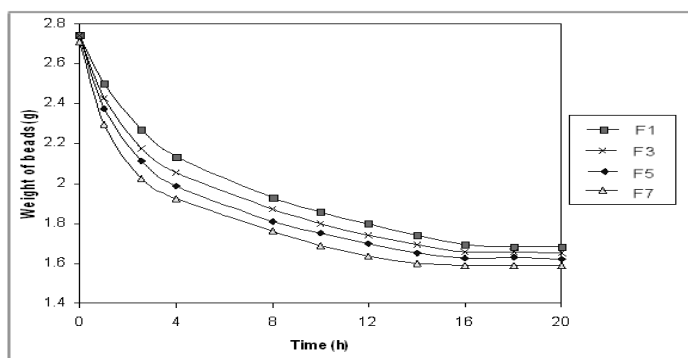


Fig. 6: Drying rate of beads (exposed to GA for 15 min)

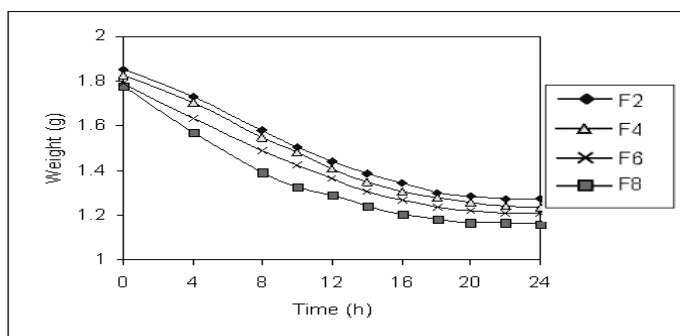


Fig. 7: Drying rate of beads (exposed to GA for 30 min)

In case of higher concentrations of chitosan, the release was comparatively slow and sustained. Both the burst effect and the continuous release phase appeared highly dependent on the concentration of polymer used for encapsulation. The crosslinking procedure gave a more sustained release in the release medium because of the denser gel structure after the crosslinking process³².³³ According to *in vitro* dissolution studies, the IPN beads showed sustained effect up to 24 hrs. The beads (exposed to GA for 15 min) containing 10 %, 30 % and 50 % alginate showed drug release of 95 %, 85 % and 80 % respectively (Fig 8). The IPN beads (exposed to GA for 30 min) containing 10 %, 30 % and 50 % alginate showed drug release of 88 %, 81 % and 77 % respectively (Fig 9). The

highest dissolution rate was obtained with F7 (95 %) whereas the lowest was F2 (73 %). Early studies reported that the drug release from the polymeric microspheres was affected by the particle size and drug: polymer ratio³⁴⁻³⁶, but in this study they showed no significant effect on the release profile of drug. Two processes can explain the release of a drug from a particle: diffusion and erosion. Drug could diffuse out of the beads, following the water phase that fills the matrix. Drug could also be released from the beads through the erosion of the matrix. Erosion could occur through the reversal of the gelation reaction, thus resulting in the solubilization of polymer molecules, or through the degradation of the polymer backbone into smaller molecular weight components³⁷.

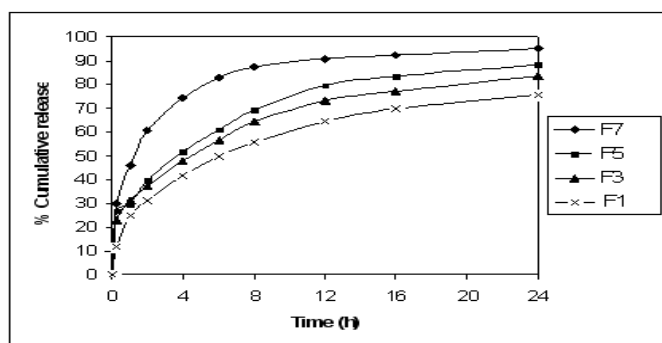


Fig. 8: *In vitro* drug release profile of IPN beads (exposed to GA for 15 min)

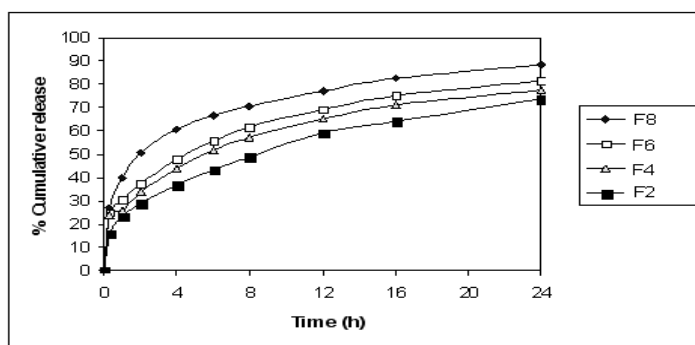


Fig. 9: *In vitro* drug release profile of IPN beads (exposed to GA for 30 min)

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